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Abstract: OBJECTIVE: Bilateral vestibular loss (BVL) is often diagnosed with great delay and an underlying cause is only identified in 50-80%. We measured horizontal and vertical semicircular canal function using the video-head-impulse test (vHIT) and hypothesized that specific vHIT-patterns may be linked to certain etiologies. METHODS: We retrospectively analyzed 109 BVL-patients linked to aminoglycoside vestibulotoxicity (n=16), Menière's disease (n=10), infectious inner-ear disorders (n=11), sensorineural hearing-loss (n=11), cerebellar-ataxia-neuropathy-vestibular-areflexia-syndrome (CANVAS, n=5), other causes (n=19) as well as those with unknown origin (n=47). Vestibulo-ocular reflex gains and cumulative saccade amplitudes were measured with vHIT, and the functional integrity of all semicircular canals was rated. RESULTS: Overall, anterior canal hypofunction (n=86/218) was identified significantly ($p<0.001$) less often than horizontal (n=186/218) and posterior (n=194/218) hypofunction. Preserved anterior canal function was associated with aminoglycoside vestibulotoxicity, Menière's disease and BVL of unknown origin, while no such sparing was found for inner-ear infections, CANVAS and sensorineural hearing loss. CONCLUSIONS: Semicircular canal function in BVL shows disease-specific dissociations, potentially related to reduced vulnerability or superior recovery of the anterior canals. SIGNIFICANCE: In patients with suspected BVL we recommend quantifying vHIT gains and saccade amplitudes for all semicircular canals as the pattern of canal hypofunction may help identifying the underlying disorder.

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Disease-specific sparing of the anterior semicircular canals in bilateral vestibulopathy

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Short title: semicircular canal hypofunction in bilateral vestibulopathy

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Highlights:

- Patterns of semicircular canal loss may indicate different etiologies in bilateral vestibulopathy
- Anterior canals were relatively preserved for aminoglycoside vestibulotoxicity and Menière's disease
- Video-head-impulse testing of all six canals may help identify bilateral vestibular loss etiologies

ABSTRACT

Objective: Bilateral vestibular loss (BVL) is often diagnosed with great delay and an underlying cause is only identified in 50-80%. We measured horizontal and vertical semicircular canal function using the video-head-impulse test (vHIT) and hypothesized that specific vHIT-patterns may be linked to certain etiologies.

Methods: We retrospectively analyzed 109 BVL-patients linked to aminoglycoside vestibulotoxicity (n=16), Menière's disease (n=10), infectious inner-ear disorders (n=11), sensorineural hearing-loss (n=11), cerebellar-ataxia-neuropathy-vestibular-areflexia-syndrome (CANVAS, n=5), other causes (n=19) as well as those with unknown origin (n=47). Vestibulo-ocular reflex gains and cumulative saccade amplitudes were measured with vHIT, and the functional integrity of all semicircular canals was rated.

Results: Overall, anterior canal hypofunction (n=86/218) was identified significantly ($p<0.001$) less often than horizontal (n=186/218) and posterior (n=194/218) hypofunction. Preserved anterior canal function was associated with aminoglycoside vestibulotoxicity, Menière's disease and BVL of unknown origin, while no such sparing was found for inner-ear infections, CANVAS and sensorineural hearing loss.

Conclusions: Semicircular canal function in BVL shows disease-specific dissociations, potentially related to reduced vulnerability or superior recovery of the anterior canals.

Significance: In patients with suspected BVL we recommend quantifying vHIT gains and saccade amplitudes for all semicircular canals as the pattern of canal hypofunction may help identifying the underlying disorder.

Keywords:

Video head impulse test, semicircular canals, bilateral vestibular loss, aminoglycosides, Menière's disease, inner-ear infection

1. INTRODUCTION

Bilateral impairment of peripheral-vestibular function is characterized by gait and postural imbalance and oscillopsia (illusionary movement of the visual surroundings during head movements) (Dandy, 1941, 1952, Hain et al. , 2013). Gait imbalance is present in up to 99% of patients (Zingler et al. , 2007), while rates of oscillopsia are more variable (range: 44% (Zingler et al. , 2007) - 97% (Black et al. , 2004)). The prevalence for severe bilateral vestibular loss (BVL) was estimated at 28/100'000 in US-adults (Ward et al. , 2013). In typical dizziness clinics, BVL is present in 2-4% of all outpatients (Zingler et al. , 2007, Hain et al. , 2013), which makes it a rather infrequent vestibular disorder. Quality of life, however, is considerably affected by BVL (Guinand et al. , 2012, Sun et al. , 2014), with >80% of patients reporting impairment (Zingler et al. , 2008). Likewise, BVL imposes substantial economic burdens on individuals and the society (Sun et al. , 2014). The course of disease is chronic in up to 80% (Zingler et al. , 2008), albeit recovery from vestibulotoxicity may occur (Black et al. , 2001).

These complaints are a consequence of a deficient angular vestibulo-ocular reflex (aVOR) that normally compensates for head perturbations. At the bedside, the head-impulse test (HIT) allows a clinical assessment of the aVOR (Halmagyi et al. , 1988). Investigations for the presence of BVL are often delayed by months to years due to unspecific complaints and lack of recognition by the clinician (Ahmed et al. , 2012, van de Berg et al. , 2015). A specific cause of BVL is found only in 50-80% of patients (Rinne et al. , 1998, Zingler et al. , 2007, Kim et al. , 2011, Lucieer et al. , 2016). Vestibulotoxic drugs such as aminoglycoside antibiotics (10-20%), bilateral Menière's disease (7-15%) and head trauma with bilateral inner-ear damage (<10%) are amongst the most frequently identified disorders linked to BVL (Vibert et al. , 1995, Rinne et al. , 1998, Zingler et al. , 2007, Kim et al. , 2011).

Traditionally, semicircular canal (SCC) function was quantified by use of caloric irrigation and rotatory-chair testing (Rinne et al. , 1998, Ishiyama et al. , 2006, Kim et al. ,

2011). These approaches, however, have significant limitations, as they only provide information about the functional state of the horizontal SCCs. Only with the recent introduction of video-based quantitative head-impulse testing (vHIT) devices, a detailed assessment of all six horizontal and vertical (anterior, posterior) SCCs became readily available (MacDougall et al. , 2009, Weber et al. , 2009b, MacDougall et al. , 2013a, b).

With different underlying disorders leading to BVL, we hypothesized that the individual pattern of SCC hypofunction may be variable. For example, in temporal-bone specimens of patients with Menière's disease (MD), varying rates of involvement of the individual SCCs and the macular organs have been reported (Okuno et al. , 1987). Potentially, characteristic patterns of SCC hypofunction will facilitate identifying the underlying cause of BVL and result in earlier and more targeted treatment, eventually improving long-term outcome. We retrospectively analyzed vHIT measurements in all patients with diagnosed BVL since the introduction of the vertical vHIT at our dizziness clinic and explored patterns of vestibular hypofunction with underlying diagnoses.

2. MATERIAL AND METHODS

The protocol was approved by the Cantonal ethics commission Zurich (KEK-ZH-2013-0468) and was in accordance with ethical standards laid down in the 2013 Declaration of Helsinki for research involving human subjects. Since this study was a retrospective database analysis, written informed consent could not be obtained from the participants. This approach was in accordance with the approval from the ethics committee. Prior to analysis patient records/information was anonymized and de-identified.

2.1 vHIT-recording procedure

The standard vHIT procedure at the University Hospital Zurich (UHZ) requires 20 valid head impulses for each canal (MacDougall et al. , 2013a), with canals tested in pairs according to the planes of stimulation (horizontal plane, right-anterior-left-posterior (RALP) plane, left-anterior-right-posterior (LARP) plane). For video-oculography, commercially available video-head-impulse testing goggles (GN Otometrics, Taastrup, Denmark) with an infrared camera recording the right eye were used. Horizontal and vertical eye position was measured with a 250Hz frame rate and head velocity was determined by three orthogonal gyroscopes. For further analysis, eye and head velocity values were calculated.

2.2 Patient identification and statistical analysis

All patients evaluated by vHIT had sought medical attention because of gait ataxia or vertigo/dizziness. The clinical database operated at the UHZ stored all vHITs performed and was searched for patients with hypofunction in at least one SCC on each side (period: October 1st 2012 to December 15th 2014), finding 110 patients out of 2430 recordings from 2123 patients. We re-analyzed aVOR-gains using OtosuiteV 2.0 (GN Otometrics). The gain of the

aVOR was calculated as the ratio of cumulative slow-phase eye velocity over cumulative head velocity from the onset of the head impulse to the moment when head velocity crossed zero again (Macdougall et al. , 2013b). For the quantification of corrective saccades we used custom-written MATLAB (The MathWorks, Natick, MA, USA) routines, providing cumulative overt saccade amplitudes (Weber et al. , 2008, 2009a). Saccades were defined as 'overt', if their onset occurred after head velocity crossed zero after the head impulse. Cumulative saccade amplitude per trial was calculated by adding all overt saccades with peak velocity >20°/s. Vestibular hypofunction was defined as a reduction in aVOR-gain and/or the occurrence of overt compensatory saccades with peak velocities >20°/s. For gains, cut-off values of 0.8 (horizontal canals) and 0.7 (vertical canals) have been proposed by the manufacturer of the video-goggles to distinguish normal from reduced aVOR-function and are in line with age-dependent normative values (McGarvie et al. , 2015).

Two experienced neuro-otologists (AAT, KPW) independently reviewed the vHIT-traces and rated individual SCC-function as normal or pathological (Cohen's kappa=0.86) (Cohen, 1960) based on 1) reduced aVOR-gain, 2) the presence of corrective saccades, or 3) a combination of both. In addition, a second rating was obtained taking the presence/absence of correction saccades into account only. BVL was confirmed in 109 cases, one patient was excluded because of unilateral vestibular hypofunction. Disagreements were settled by discussion amongst the reviewers. The underlying cause of BVL was retrieved from the patients' files. We followed the AAO-HNS 1995 guidelines for diagnosing MD (1995). Details on previous CNS-infections, drug treatment and delay for vestibular symptoms were collected (Appendix A). A diagnosis of bilateral sensorineural hearing loss (SNHL) required documented hearing impairment as assessed by pure tone audiogram based on CPT-AMA guidelines (Council on Physical Therapy, 1942) with a CPT value >20% on both sides and exclusion for other causes. Diagnostic criteria for CANVAS (cerebellar ataxia, neuropathy, vestibular areflexia syndrome) were based on the definition provided by Szmulewicz

(Szmulewicz et al. , 2011). The individual patterns of vestibular (hypo)function and the reported cause of BVL were correlated and an analysis of the occurrence of specific patterns was made. In those cases with both warm-/cold-water caloric irrigation and vHIT available (n=66), these two approaches were compared. Unilateral hypofunction was defined as a canal paresis factor of >25% with a preserved response on the healthy side (Halmagyi et al. , 1997), while for bilateral hypofunction on caloric irrigation a nystagmus with a mean peak slow-phase velocity of less than 5°/sec on both sides was required (Zingler et al. , 2007).

MATLAB was used for statistical analysis. Fisher's exact test (Bonferroni-corrected) was applied to determine significant differences in the frequency of specific conditions. Median values (±1 median absolute deviation, MAD) were determined for gains and saccade amplitudes and statistics were based on non-parametric analysis of variance (Kruskal-Wallis ANOVA, Tukey-Kramer corrected). The level of significance for all statistical tests was p=0.05. Gain asymmetry was determined using the absolute gain difference between both sides as this approach avoids exaggerated asymmetry in patients with very small aVOR-gains (Weber et al. , 2009a).

Currently no established cut-off values are available to identify pathological correcting saccades after a head-impulse. We therefore obtained a ROC (Receiver Operating Characteristic) curve to estimate the optimal cut-off value for cumulative saccade amplitudes based on the reviewers' ratings and the saccade amplitudes using the built-in MATLAB function perfcurve.m. The closer the ROC curve approaches the top left-hand corner (100% sensitivity and 100% specificity) the more accurate is the test. The accuracy of the test is determined by the area under the curve (AUC).

2.3 Assessment of diagnostic patterns

In exploratory analyses we looked at the strength of association between gain and amplitude parameters and the underlying etiology for BVL. As reference subgroups we

198 selected aminoglycoside-vestibulotoxicity and MD. We pooled gains and amplitude
199 parameters of both sides into horizontal, anterior and posterior parameters for gain and
200 amplitude, respectively.

201 The strength of association of horizontal SCC, anterior SCC and posterior SCC
202 (independent variates) for each of two etiologies (dependent variate) was examined as
203 follows: In context of aminoglycoside-related BVL, 1) SNHL was compared to
204 aminoglycosides, 2) infectious was compared to aminoglycosides and 3) MD was compared
205 to aminoglycosides. In the context of MD-related BVL, 4) SNHL was compared to MD and
206 5) infectious was compared to MD. Analyses were performed for gain and cumulative
207 saccade amplitude separately resulting in 10 different models. The intercept was set to 0. With
208 these models, we estimated the odds ratios (OR) and 95% confidence-intervals (CI) for each
209 of the three gain and amplitude parameters for each of the etiology contrasts. Due to the
210 exploratory nature of this analysis, we refrain from reporting p-values between different
211 groups of diagnoses. Analyses were performed using Stata 14.0 (StataCorp LP, College
212 Station, TX).
213

3. RESULTS

One-hundred-nine BVL cases (47 females, 63.8±17.9 years, mean±1SD) were included. Most frequent causes of BVL were vestibulotoxic drugs (15%), SNHL (10%) and inner-ear infections (10%) (Table 1 and appendix A).

3.1 Distribution of affected SCCs

Peripheral-vestibular hypofunction was identified in 466/654 SCCs (71.3%). There was no significant ($p>0.05$, Fisher's exact test) preference of the side affected. While percentages (left and right side pooled) of horizontal (n=186/218, 85.3%) and posterior (n=194/218, 89.0%) SCCs with hypofunction were similar ($p=0.94$), anterior canals were impaired significantly ($p<0.001$) less often (n=86/218, 39.5%) (Figures 1 and 2).

/*Figures 1&2 about here*/

We correlated the combination of affected SCCs with the underlying clinical diagnosis, but restricted this analysis to disorders with a sample size ≥5 to allow for statistical analysis of these subgroups. Rates were significantly higher for the horizontal and posterior canals compared to the anterior canals in patients with aminoglycoside-related vestibulotoxicity, MD and BVL of unknown origin (Table 2 and Figure 2C). From the 66 cases with caloric irrigation available, lateral SCC function (vHIT vs. calorics) matched in 40 cases (60.6%). In half of the discordant cases (13/26), bilateral SCC hypofunction in the vHIT contrasted with a significant asymmetry ratio in calorics; in six cases calorics were normal despite bilateral vHIT hypofunction. In the remaining seven cases, unilateral hypofunction was noted in only one of the two tests.

3.2 Analysis of SCC-gains

Reduced gains were found in 438/654 SCCs (67.0%) (Figure 3A). Rates were significantly ($p<0.001$) higher for posterior (n=165/218) and horizontal (n=175/218) canals than for anterior (n=98/218) canals. Median (±1MAD) absolute gain asymmetries were 0.13±0.08 (horizontal canals), 0.13±0.09 (anterior canals), and 0.11±0.08 (posterior canals), respectively. With no significant lateralization of gains in either plane, further analyses were done after pooling gains from both sides. While median gains for anterior canals were significantly higher than those for the horizontal (0.73 vs. 0.54, $p<0.001$) and posterior (0.73 vs. 0.50, $p<0.001$) SCCs, there was no significant difference (0.54 vs. 0.50, $p=0.480$) between horizontal and posterior canals (Figure 3B).

/*Figure 3 about here*/

In relation to the overall rating of SCC function (based on the assessment by two experienced neuro-otologists), we calculated the sensitivity and specificity of obtained aVOR gain values based on currently accepted gain cut-offs for hypofunction (<0.8 for the horizontal canals; <0.7 for the vertical canals). Both sensitivity (87.6% [95%-CI: 84.6-90.6]) and specificity (84.0% [78.8-89.6]) were high.

We correlated SCC gains with specific disorders. In accordance with the clinical rating of canal function, median anterior canal gains were significantly ($p<0.001$) larger than those of posterior and horizontal canals (Table 2) for aminoglycoside-related vestibulotoxicity and BVL of unknown origin. Likewise median gains in Menière-related BVL were significantly higher for anterior canals compared to posterior canals ($p=0.002$), but not significantly ($p=0.166$) different between anterior and horizontal canals (Figure 4). For BVL related to CANVAS, SNHL and infections, no significant gain differences between anterior, posterior and lateral canals were found.

/*Figure 4 about here*/

3.3 Analysis of compensatory saccades

Cumulative saccade amplitudes were rated as abnormally high in 432/654 canals (66.1%). Percentages were significantly ($p<0.001$) higher for horizontal (n=181/218, 83.0%) and posterior (n=184/218, 84.4%) canals than for anterior canals (n=67/218, 30.7%). In all subgroups (Table 2) correction saccades were identified significantly ($p<0.05$) more often for horizontal than for anterior SCCs. When comparing the anterior and posterior SCCs, correction saccades were more common in posterior canals only for aminoglycoside-related vestibulotoxicity, Menière-related BVL and BVL of unknown origin.

We did not observe significant lateralization of saccade amplitudes (Figure 3C). Pooled amplitudes (Figure 3D) for horizontal canals were significantly higher than those for posterior (2.46 vs. 1.48°/trial, $p=0.001$) and anterior (2.46 vs. 0.28°/trial, $p<0.001$) canals. Likewise amplitudes for posterior canals were significantly larger than those for anterior canals (2.46 vs. 0.29°/trial, $p<0.001$). Median amplitudes (Table 2) of both horizontal and posterior canals were significantly ($p<0.01$) larger than those of anterior canals for aminoglycoside-related vestibulotoxicity, BVL of unknown origin and MD (Figure 4). For CANVAS, SNHL and inner-ear infections, median saccade amplitudes of horizontal SCCs were also significantly larger than those of anterior canals ($p<0.05$), while no significant difference was found when comparing anterior and posterior canals.

With both gain reduction and correction saccades emerging from SCC hypofunction, we predicted a substantial inverse correlation between these two parameters. When plotting gains against saccade amplitudes (Figure 3E), we indeed noted a significant ($p<0.001$) inverse relationship, which could be fitted significantly ($p=0.019$) better with an exponential-decay function ($R^2=0.43$) than with a linear function ($R^2=0.38$).

While the largest fraction of hypofunctional SCCs (n=466) had both reduced gains and significant correction saccades (n=375, 80.5%, based on visual inspection by experienced neuro-otologists), we found SCCs with hypofunction but absent saccades (n=35, 7.5%) and SCCs with significant correction saccades but normal gain (n=56, 12.0%). Cumulative saccade amplitudes in SCCs rated hypofunctional were significantly ($p<0.001$) smaller in those SCCs with normal gain than in those with reduced gain (Figure 3F). Likewise gain values in those SCCs with overall function rated as normal were significantly larger than in those SCCs with significant correction saccades but normal gain (0.90±0.14 vs. 0.82±0.07, $p=0.027$, Kruskal-Wallis ANOVA).

In order to determine the optimal cut-off value for abnormally increased cumulative saccade amplitudes based on the reviewers' ratings and the saccade amplitudes (see methods section for details), a ROC curve for cumulative saccade amplitudes was calculated. With an area-under-the-curve (AUC) value of 0.94, an optimal cut-off value for cumulative saccade amplitudes (sensitivity=86.5% [95%-CI=83.4-89.6]; specificity=92.6% [88.8-96.3]) of 0.73°/trial (Figure 5A) was obtained. This cut-off value allowed a reliable distinction between canals rated as having normal (left column) or reduced (right column) function for cumulative saccade amplitudes (Fig. 5B).

/*Figure 5 about here*/

Comparing the sensitivity and specificity of aVOR gains (with pre-specified cut-off values of 0.8 for horizontal SCCs and 0.7 for vertical SCCs) and cumulative saccade amplitudes (using the cut-off value of 0.73°/trial as determined), no significant differences in the sensitivity could be observed (OR=0.91 [0.62-1.33]; gains vs. cumulative saccade amplitudes). For specificity, however, risk for a false positive diagnosis was significantly

316 higher when considering gain values than for cumulative saccade amplitudes (OR=2.36 [1.21-
317 4.61]).

318 In a next step we asked whether an assessment based on both parameters would further
319 improve sensitivity and specificity. We therefore combined the ratings based on aVOR gains
320 and on cumulative saccade amplitudes in such a way that a given SCC was considered
321 hypofunctional if either both or only one of the two ratings yielded an abnormal value (i.e.,
322 gain below cut-off, cumulative saccade amplitude above cut-off). Sensitivity derived from
323 this combined assessment (96.8% [95.2-98.4]) was significantly higher than sensitivity solely
324 assessed by aVOR gain (OR=4.27 [2.39-7.66]) or cumulative saccade amplitudes (OR=4.70
325 [2.63-8.39]). Specificity obtained by pooling both assessments (80.3% [74.6-86.0]) was not
326 worse than aVOR-gain based specificity (OR=0.77 [0.46-1.32]). However, compared to
327 sensitivity derived from cumulative saccade amplitudes, specificity in the pooled assessment
328 was significantly lower (OR=0.33 [0.17-0.63]).
329

330 **3.4 Multivariate logistic regression analysis**

331 In comparison to cases with aminoglycoside-related BVL, the gain (OR=0.05 [95%-
332 CI=0.00-0.95]) and saccade amplitude (OR=2.62 [0.98-7.00]) of anterior SCCs in cases with
333 SNHL made a distinction, with SNHL being very unlikely in case of normal SCC-gain and
334 small cumulative saccade amplitudes. For aminoglycoside-related BVL vs. BVL linked to
335 inner-ear infection, again the gain (OR=0.20 [0.02-1.69]) and the saccade amplitude
336 (OR=2.91 [1.09-7.75]) of the anterior SCC contributed the most in distinguishing these
337 etiologies, favoring aminoglycoside-vestibulotoxicity over inner-ear infection for normal
338 anterior gains and saccade amplitudes. For the other subgroups, ORs for gain and saccades
339 did not help in separating the different disease entities as 95%-CI were very broad including
340 1.0 (appendix B).

4. DISCUSSION

We found striking differences in the pattern of SCC hypofunction in patients with BVL. While anterior SCC function was relatively spared in aminoglycoside-vestibulotoxicity, MD and BVL of unknown origin, SCC hypofunction was evenly distributed among all canals in BVL related to SNHL, inner-ear infections and CANVAS. Gain-reductions (reflecting aVOR-impairment) and emerging compensatory saccades (triggered by retinal slip) were highly correlated, strongly supporting the assessment of both parameters to identify peripheral-vestibular hypofunction. Noteworthy, both approaches have their advantages and disadvantages, which is reflected in the observation that 12% of SCCs had increased cumulative saccade amplitudes but still normal gains. Noteworthy, the use of cumulative saccade amplitudes as a parameter of peripheral-vestibular hypofunction has been so far limited by lack of established cut-off values. Based on ROC curves we found an optimal cut-off value of 0.73°/trial for cumulative saccade amplitudes in our patient cohort with bilateral vestibular loss. For this cut-off we achieved high sensitivity (86.5%) and specificity (92.6%). Noteworthy, this value matches very well the observed cut-off of 0.78°/trial in a small case-control series with patients with unilateral and bilateral vestibulopathy published very recently (MacDougall et al. , 2016).

The significantly higher specificity when considering cumulative saccade amplitudes instead of gain values (OR=2.36) further underlines the advantages of taking cumulative saccades into account in the overall assessment. Thereby false positive ratings by erroneously low gain values (e.g. due to slippage of the vHIT goggles) may be avoided. As a result, combining both aVOR-gain based and saccade-based ratings significantly increased the sensitivity of the vHIT (92.4%) compared to the use of a single parameter only, while specificity remained high (being not worse than for ratings based on aVOR gains solely).

366 **4.1 Disease-specific sparing of the anterior SCCs**

367 Considering the disease-specific sparing of the anterior SCCs, vHIT may allow the
368 distinction between BVL related to aminoglycosides or MD and BVL associated with inner-
369 ear infections or SNHL. This is underlined by logistic regression analysis: both gains and
370 saccade amplitudes of anterior SCCs helped in distinguishing between aminoglycoside-
371 related BVL and BVL secondary to SNHL or inner-ear infections. Anterior SCC-sparing was
372 noted also in BVL of unknown origin. It is reasonable to assume that aminoglycoside-
373 vestibulotoxicity and MD may be underdiagnosed and they potentially make up a significant
374 fraction of all BVL cases of unknown origin.

375 With regards to cumulative saccade amplitudes, disease-specific sparing of anterior
376 SCC function was found only when comparing anterior and posterior SCC function, whereas
377 amplitudes of horizontal SCCs were significantly higher in all subgroups compared to those
378 of the anterior SCCs. These differences can be explained by the way the head-impulse-test is
379 performed: due to biomechanical reasons, horizontal head impulses are applied with
380 somewhat larger amplitudes than vertical head impulses. Therefore, resulting correction
381 saccades in case of SCC hypofunction will be larger for the horizontal than for the vertical
382 SCCs, while the vertical canals are always tested in pairs (LARP and RALP planes), allowing
383 direct comparison between anterior and posterior SCCs. For the analysis of cumulative
384 saccade amplitudes, sparing of anterior SCC function is a disease-specific marker only when
385 compared to posterior SCC function.

386 Previously, Agrawal and colleagues reported oVEMPs to be the most sensitive test to
387 distinguish between aminoglycoside-related vestibulotoxicity and bilateral MD with the latter
388 disease showing relative sparing of oVEMP-function compared to the first one (Agrawal et al.
389 , 2013). Since SCC function was only assessed using calorics by Agrawal and colleagues and
390 since we did not incorporate oVEMP-measurements here, this leaves the question open, to
391 which extent differences in anterior/horizontal SCC correlate to otolith-function.

392 Anterior SCC-sparing may originate from different causes, including distinct
393 vulnerability of ampullary hair cells to vestibulotoxic substances or increased endolymphatic
394 pressure and superior recovery after damage.

395 Aminoglycosides are hydrophilic drugs with low protein-binding and a molecular
396 weight between 450 and 600g/mol. They accumulate in the endolymph and perilymph (Tran
397 Ba Huy et al. , 1983), readily penetrate the hair cells and accumulate with a long second half
398 life (gentamicin about 30 days) (Tran Ba Huy et al. , 1986). After systemic aminoglycoside
399 administration dose dependent toxicity in the vestibular hair cells - especially in the
400 epithelium of the cristae ampullaris (Lindeman, 1969, Nakagawa et al. , 1998, Tsuji et al. ,
401 2000) was observed. Pathophysiologically, aminoglycosides are believed not to be toxic
402 unless chelated to a metal ion and subsequent generation of reactive oxygen species, cell
403 apoptosis and necrosis (Hoch et al. , 1998). A large proportion of hair cells were affected in
404 the ampulla and in the utricule (Aran et al. , 1982). Most results were derived from animal
405 studies (e.g. guinea pigs) where anatomic differences to humans may account for
406 discrepancies in susceptibility to ototoxicity. However, to our knowledge, there is no
407 confirmation of preferential damage of the posterior and horizontal canals in animal models
408 after systemic application of aminoglycosides. Three weeks after intratympanic application of
409 gentamicin in chinchilla, rates of type 1 hair-cell density reduction were similar in the
410 ampullae of horizontal and vertical canals (range=63-73%) (Lyford-Pike et al. , 2007), not
411 supporting the hypothesis of selective damage of type 1 hair cells in posterior and horizontal
412 SCCs.

413 Theoretically, accumulation of the drug may be influenced by the pull of gravity.
414 Noteworthy, basal outer cochlear hair cells were reported to be more vulnerable than apical
415 outer hair cells (Tan 2001), which could be related to basal accumulation of aminoglycosides.
416 As the posterior and horizontal canals are located below the anterior canal when in a
417 horizontal body position (as typically experienced in severely-ill patients treated with

aminoglycosides), accumulation of aminoglycosides at the lowest point would result in higher concentrations in the posterior and horizontal SCCs. A prerequisite for this hypothesis related to gravity is that the molecular density of aminoglycosides is higher than that of endolymph. A complexation and chelation with metal ions could also lead to decreased solubility and subsidence within the lymph fluid along gravitation.

Histopathological studies reporting on the relative frequency of hydrops of the otolith organs and individual SCCs are rare. In a study on temporal-bone specimens of patients with MD, hydrops was reported less frequently for the SCCs (n=10/22) compared to the otolith organs (n=19/22) (Okuno et al. , 1987). Differences in the rate of affected posterior (n=8), anterior (n=6) and horizontal (n=6) canals were non-significant, though the small sample size gave the test low power. How SCC hypofunction secondary to endolymphatic hydrops in MD therefore occurs at distinct rates for the individual SCCs remains unclear. Imaging of structural changes of the vestibular organs using MRI is a recent diagnostic approach in patients with suspected MD. Reduced or absent perilymphatic enhancement after intratympanic gadolinium application was used as an indirect sign of endolymphatic hydrops, reporting larger fractions of affected canals for anterior SCCs than for horizontal SCCs (62% vs. 46%) (Fiorino et al. , 2011). This finding is opposite to the relative sparing of anterior SCCs described here. However, whether structural changes on the level of individual SCCs correlate with functional SCCs deficits has not been evaluated.

While in the evolution of the vertebrate labyrinth (see (Carey et al. , 2006)) the original pair of vertical SCCs (as in the lamprey (Lowenstein et al. , 1968)) was complemented later by a third, horizontal SCC that develops after the vertical canals (Sher, 1971) and grows at a slower rate (Jeffery et al. , 2004), this does not explain relative sparing of anterior canals in aminoglycoside-related vestibulotoxicity and MD. Theoretically, anterior SCC sparing could be a measurement bias. This, however, seems unlikely for several reasons. First, sparing was restricted to certain disorders. Second, the vertical SCCs are always tested

444 in pairs according to their planes of stimulation and head impulses of similar velocities were
445 applied for all vertical SCCs. Third, anecdotally, patients reported oscillopsia for upward
446 head-movements only, but not for downward head movements, matching their vHIT-pattern
447 of spared anterior SCCs.
448

449 **4.2 Limitations**

450 There is no other established test to assess SCC-function in such detail as the vHIT
451 does, limiting the validation process of vHIT results. Both the established gold standard
452 (caloric irrigation) and rotatory chair testing are limited to the horizontal SCCs and magnetic
453 search coils (see e.g. (Weber et al. , 2009a)) are available only in few specialized vestibulo-
454 ocular motor laboratories. Caloric irrigation was available only in 61% of patients studied.
455 The largest fraction of discordant ratings (50%) was related to a significant asymmetry ratio
456 in calorics while the vHIT indicated bilateral hypofunction. Noteworthy, a significant canal
457 paresis factor in calorics does not necessarily implicate normal SCC-function on the side with
458 the larger response; it only indicates different levels of SCC-function on both sides.
459 Importantly, unilateral vestibular hypofunction is often accompanied by compensatory down
460 regulation of the aVOR gain and catch-up saccades on the healthy side (Weber et al. , 2008),
461 which may mimic bilateral (asymmetric) vestibular hypofunction. Such misclassification by
462 our reviewers cannot be fully excluded, however, considering the low range of gain
463 asymmetries in pairs of SCCs rated as bilaterally abnormal (0.11±0.10, median±1MAD), this
464 seems unlikely.

465 In order to determine an optimal cut-off value for cumulative saccade amplitudes in
466 affected semicircular canals, the qualitative ratings of two expert neuro-otologists were
467 considered as reference standard. Obviously, this bears the risk of inter-observer variability,
468 however, ratings were independently obtained and discrepancies were settled by discussion.
469 Whether the cut-off value obtained (0.73°/trial) is valid also for other peripheral-vestibular

disorders (e.g. acute unilateral vestibular neuropathy), however, is subject to future (ideally prospective) studies.

With the underlying cause of BVL remaining unclear in 47 cases, both labyrinthine and retro-labyrinthine disorders may be considered. Brain imaging (MRI in 38 cases; CT in 3 cases) was available in 41 cases, demonstrating definite unilateral retro-labyrinthine pathologies in only 2 cases (both vestibular schwannoma) while no pathologies along the vestibular nerve were described in the remaining 39 cases. Overall, imaging does not provide a convincing explanation for the bilateral SCC hypofunction in any of the unclear cases that received imaging (87.2%). Furthermore, with presumed retro-labyrinthine pathology, rather a pattern of posterior canal sparing (in case of superior branch vestibular neuropathy), anterior and horizontal canal sparing (in case of inferior branch vestibular neuropathy) or involvement of all SCCs (e.g., in case of vestibular schwannoma) would be predicted based on the innervation and vascularization of the vestibular organ. Regarding the small fraction of cases with confirmed retro-labyrinthine disorders (3 cases, mostly vestibular schwannoma), no anterior canal sparing was observed in these cases.

5. CONCLUSIONS

Peripheral-vestibular mapping with vHIT including all six SCCs may be considered in patients with suspected BVL for differentiating between distinct disease etiologies. Sparing of anterior SCCs may point to aminoglycoside-related vestibulotoxicity or MD. With 12% of cases showing correction saccades but normal gain values, we propose to include saccade amplitudes into the overall rating of canal function to increase sensitivity. We could not identify any morphological, functional or developmental differences between the anterior and posterior SCCs that may provide a convincing explanation for disease-specific anterior-SCC sparing. Mechanistic models investigating effects of aminoglycoside- or MD-related toxicity in the more caudally located SCCs (i.e., the lateral and posterior canals) should clarify preferential damage location in further studies.

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609

FIGURE LEGENDS

Figure 1:

Video-head-impulses from a single patient demonstrating bilateral vestibular deficits related to aminoglycoside-induced vestibulotoxicity. Eye velocity traces (in black) and head velocity traces (in grey) are plotted against time. Peripheral-vestibular hypofunction was diagnosed here for both horizontal and both posterior SCCs based on reduced gains (<0.8 for the horizontal SCCs; <0.7 for the vertical SCCs; as also shown in the center hexplot) and increased cumulative saccade amplitudes. In this patient the anterior SCCs were bilaterally spared.

Figure 2:

Summary figures on the distribution of peripheral-vestibular hypofunction in our 109 patients, illustrating both the fractions with hypofunction for the different SCCs as a hexplot (panel A) and the relative frequency of specific combinations of SCC hypofunction (panel B). *Diverse combinations included one case each with SCC hypofunction bilateral anterior unilateral horizontal, bilateral anterior unilateral posterior, bilateral horizontal unilateral anterior and horizontal, bilateral anterior and horizontal unilateral posterior, bilateral posterior unilateral anterior and horizontal, bilateral anterior, unilateral horizontal & anterior (left side) and unilateral posterior (right side). Panel C: Comparison of the relative frequency of peripheral-vestibular SCC hypofunction in different planes (based on the reviewers ratings) and for distinct underlying disorders (note that only subgroups with an n≥5 were considered). This value is provided as a ratio calculated by dividing the fraction of anterior SCCs with hypofunction by the pooled and averaged fraction of horizontal and posterior SCCs with hypofunction. Here this ratio is plotted against the number of patients with this diagnosis, showing significant anterior SCC-sparing in aminoglycoside-related BVL, MD and BVL of

635 unknown origin (ratio=0.2-0.3), but not in the other subgroups (ratio=0.8-0.9). For infection-
636 related BVL and CANVAS the ratio was identical (0.8), therefore these subgroups were
637 plotted above each other.
638 Abbreviations: SNHL= sensorineural hearing-loss; LA=left anterior SCC; LH=left horizontal
639 SCC; LP=left posterior SCC; RA=right anterior SCC; RH=right horizontal SCC; RP=right
640 posterior SCC.
641

642 **Figure 3:**

643 Comparison of gain values (panels A and B) and cumulative saccade amplitudes
644 (panels C and D) in the BVL patients. Panel A: A hexplot is used to illustrate the distribution
645 of median (±1 MAD) gain values for all six SCCs separately. Since statistical analysis
646 (Tukey-Kramer corrected for multiple comparisons) did not find effects of laterality, gain
647 values from corresponding SCCs on the left and right side were pooled, as shown in panel B
648 including all 654 canals. Panel C: Hexplot illustrating the cumulative saccade amplitudes for
649 all six SCCs separately in all BVL patients. Again, values from corresponding left and right
650 SCCs were pooled for further analysis, as shown for all 654 SCCs in panel D. Panel E: Gain
651 values are plotted against the cumulative saccade amplitudes for all canals and patients tested,
652 demonstrating a significant inverse correlation for both fits. In comparison, the fit of the
653 exponential decay function is significantly better than the linear fit ($p=0.019$, F-test). Panel F:
654 comparison of cumulative saccade amplitudes for trials rated as having peripheral-vestibular
655 hypofunction but normal (left column) or reduced (right column) gain. In the box and whisker
656 plots used for panels B, D, and F the box has lines at the lower quartile, the median and the
657 upper quartile values. Notches represent the 95% confidence interval around the median.
658 Whiskers extend from each end of the box, including data points within approximately 2.7
659 standard deviations of the median. Outliers („+“ signs) are data with values beyond the ends
660 of the whiskers. * indicates statistically significant differences ($p<0.05$) based on non-

parametric Kruskal-Wallis ANOVA and multiple comparisons with Tukey-Kramer correction, ns refers to statistically non-significant differences. For explanation of other abbreviations see legend of Figure 2.

Figure 4:

Hexplots illustrating the median gain values (±1MAD) and the cumulative saccade amplitude for all six SCCs separately in the most frequent diseases linked to BVL observed in our study population. While the anterior canals yielded higher gains and lower saccade amplitudes for aminoglycoside-related BVL (panel A), BVL of unknown origin (panel B) and BVL related to Menière's disease (panel C), gain values for all six canals were reduced in a similar fashion for CANVAS (panel D), sensorineural hearing-loss (SNHL, panel E) and BVL linked to inner-ear infections (panel F). The same picture was found for the distribution of cumulative saccade amplitudes (panels G-L).

Figure 5:

Panel A: Receiver operating characteristic (ROC) curve analysis for an optimal cut-point of cumulative saccade amplitudes to detect a pathological video-head-impulse test. The closer the ROC curve approaches the top left-hand corner (100% sensitivity and 100% specificity) the more accurate is the test. The accuracy of the test is determined by the area under the curve (AUC), reaching 0.94 for cumulative saccade amplitudes here. Noteworthy, a perfect test has an AUC=1, whereas a worthless test such as the hypothetical reference diagonal line has an AUC=0.5. The stars indicate the optimal cut-off point for cumulative saccade amplitudes. In panel B cumulative saccade amplitudes for canals judged as normal are compared with those rated as abnormal with the dashed black line indicating the optimal cut-off point (cumulative saccade amplitude=0.73°/trial). The grey dots refer to individual

686 canals. In the box and whisker plots used for panel B the box has lines at the lower quartile,
687 the median and the upper quartile values. Whiskers correspond to approximately ±2.7σ of the
688 median. Outliers („+“ signs) are data with values beyond the ends of the whiskers.

689 **TABLES**

***Table 1**: epidemiological findings of the 109 patients with BVL*

		SCCs with hypofunction (%)					
Disease	**Cases (%)**	**LE hor**	**RE hor**	**LE ant**	**RE ant**	**LE post**	**RE post**
Unknown	47 (43.1)	39 (83.0)	39 (83.0)	12 (25.5)	11 (23.4)	41 (87.2)	44 (93.6)
Vestibulotox. drugs ‡	16 (14.7)	16 (100)	14 (87.5)	5 (31.3)	4 (25.0)	16 (100)	16 (100)
Bilateral SNHL	11 (10.1)	10 (90.1)	10 (90.1)	9 (81.8)	7 (63.6)	10 (90.1)	10 (90.1)
Infectious	11 (10.1)	11 (100)	9 (81.8)	8 (72.7)	8 (72.7)	9 (81.8)	9 (81.8)
Menière's disease	10 (9.2)	6 (60.0)	5 (50.0)	2 (20.0)	1 (10.0)	6 (60.0)	9 (90.0)
CANVAS	5 (4.6)	5 (100)	5 (100)	4 (80.0)	4 (80.0)	5 (100)	5 (100)
Autoimmune *	2 (1.8)	2 (100)	2 (100)	2 (100)	1 (50.0)	2 (100)	2 (100)
Bilat. schwannoma	2 (1.8)	2 (100)	2 (100)	1 (50.0)	2 (100)	1 (50.0)	2 (100)
Head trauma	2 (1.8)	2 (100)	1 (50.0)	1 (50.0)	1 (50.0)	2 (100)	2 (100)
Central causes †	1 (0.9)	1 (100)	1 (100)	1 (100)	0 (0.0)	0 (0.0)	1 (100)
Schwannoma + VN §	1 (0.9)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)
Fabry disease	1 (0.9)	1 (100)	1 (100)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Total	**109 (100)**	**96 (88.1)**	**90 (82.6)**	**46 (42.2)**	**40 (36.7)**	**93 (85.3)**	**101 (92.7)**

690

691 Abbreviations: ant=anterior; bilat=bilateral; CANVAS=cerebellar ataxia with neuropathy and

692 bilateral vestibular areflexia syndrome; hor=horizontal; LE=left; post=posterior; RE=right;

693 SNHL=sensorineural hearing loss; VN=vestibular neuritis

694

695 * One case of possible Cogan's syndrome, one case with autoimmune-related disorder

696 possibly associated with neurosarcoidosis.

697 † MRI-confirmed cavernoma in the left brachium pontis with possible involvement of the

698 vestibular nuclei.

699 ‡ This includes the following aminoglycosides: Gentamicin (n=12), Tobramicin (n=2) and

700 Streptomycin (n=1). In one case the type of aminoglycoside remained unclear.

701 § Sequential occurrence of vestibular schwannoma on one side and vestibular neuritis on the

702 other side (n=1).

***Table 2**: Subgroup analyses for gain and saccades*

Reviewers' ratings of SCC function

	global ratings (based on gain and correction saccades)						**ratings based on correction saccades only**					
	SCC hypofunction (left / right side pooled)			*p-value (Fisher's exact test*)*			*significant correction saccades (left / right side pooled)*			*p-value (Fisher's exact test*)*		
Disease	hor SCC	ant SCC	post SCC	hor vs. ant	hor vs. post	ant vs. post	hor SCC	ant SCC	post SCC	hor vs. ant	hor vs. post	ant vs. post
Unknown	78/94	23/94	85/94	**<0.001**	0.197	**<0.001**	74/94	17/94	79/94	**<0.001**	0.454	**<0.001**
Aminoglycosides	30/32	9/32	32/32	**<0.001**	0.585	**<0.001**	30/32	7/32	32/32	**<0.001**	0.585	**<0.001**
SNHL	20/22	16/22	20/22	0.241	1.000	0.241	20/22	13/22	18/22	**0.034**	0.655	0.185
Infectious	20/22	16/22	18/22	0.241	0.655	0.721	20/22	13/22	18/22	**0.034**	0.655	0.185
Menière	11/20	3/20	15/20	**0.019**	0.320	**<0.001**	11/20	3/20	14/20	**0.019**	0.515	**0.001**
CANVAS	10/10	8/10	10/10	0.540	1.000	0.540	10/10	5/10	10/10	**0.034**	1.000	**0.034**

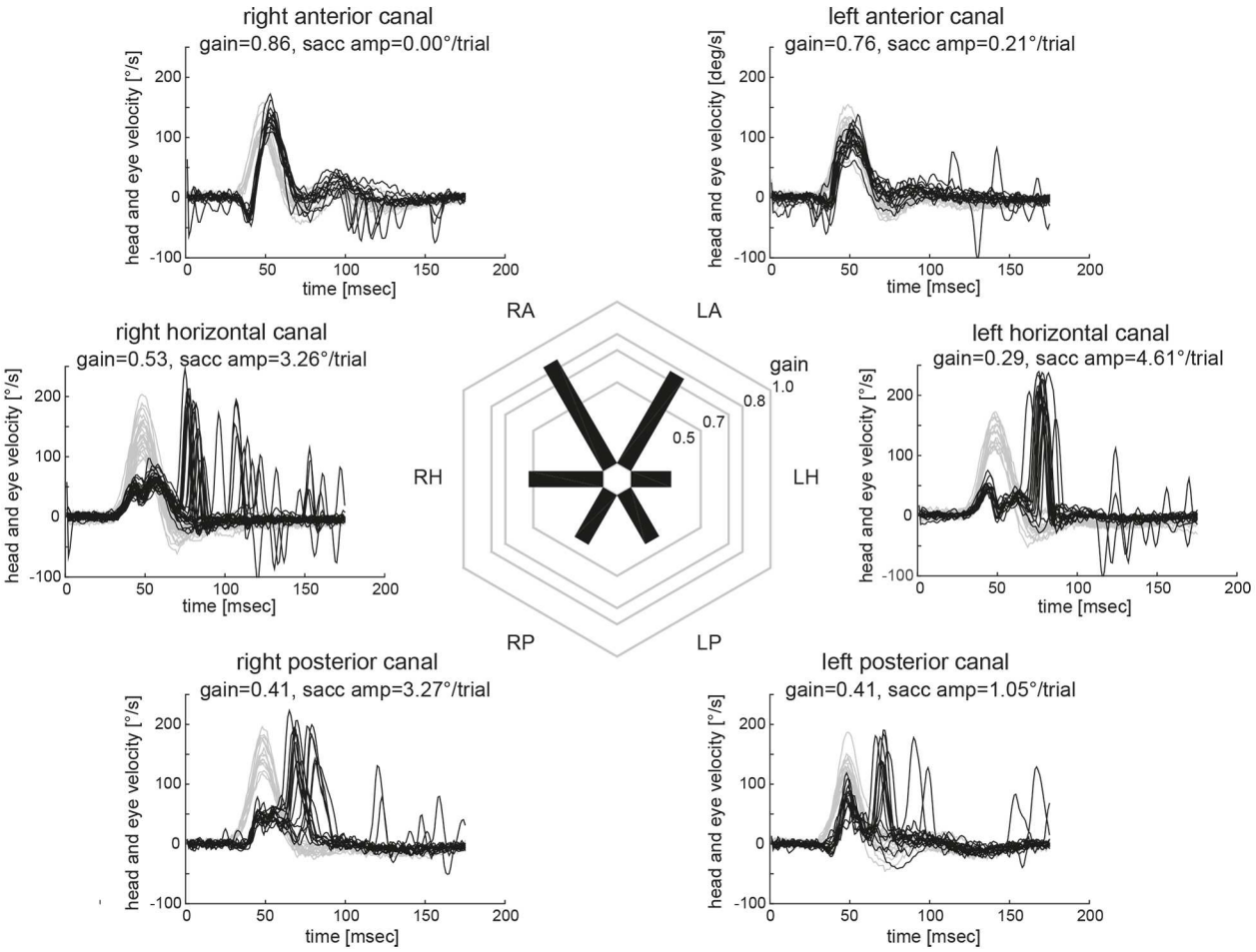
Measured gains and cumulative saccade amplitudes

	vHIT gains						**correction saccades**					
	gain value (median±1MAD)			*p-value (Kruskal-Wallis ANOVA†)*			cumulative saccade amplitude [°/trial] *(median±1MAD)*			*p-value (Kruskal-Wallis ANOVA†)*		
Disease	hor SCC	ant SCC	post SCC	hor vs. ant	hor vs. post	ant vs. post	hor SCC	ant SCC	post SCC	hor vs. ant	hor vs. post	ant vs. post
Unknown	0.58±0.19	0.82±0.16	0.58±0.18	**<0.001**	0.722	**<0.001**	2.33±1.14	0.20±0.20	1.58±0.94	**<0.001**	0.049	**<0.001**
Aminoglycosides	0.46±0.17	0.77±0.15	0.41±0.12	**<0.001**	0.961	**<0.001**	2.66±1.29	0.38±0.34	1.85±0.78	**<0.001**	0.350	**<0.001**
SNHL	0.47±0.26	0.61±0.23	0.53±0.20	0.500	0.994	0.564	1.60±1.20	0.47±0.40	1.36±0.89	**0.004**	0.136	0.411
Infectious	0.36±0.21	0.48±0.15	0.28±0.09	0.302	0.972	0.204	3.41±0.92	1.59±1.19	1.71±1.14	**0.004**	0.078	0.544
Menière	0.76±0.17	0.99±0.11	0.61±0.13	0.166	0.248	**0.002**	1.01±0.77	0.02±0.02	1.16±0.35	**<0.001**	0.629	**0.008**
CANVAS	0.13±0.07	0.31±0.17	0.30±0.13	0.137	0.442	0.772	4.42±0.68	0.58±0.58	2.15±1.08	**0.012**	0.503	0.195

Abbreviations: ant=anterior; CANVAS=cerebellar ataxia with neuropathy and bilateral vestibular areflexia syndrome; SNHL=sensorineural hearing-loss, hor=horizontal: MAD=median absolute deviation; post=posterior; SCC=semicircular canal; vHIT=video-head-impulse test

* With Bonferroni correction for multiple tests.

† With Tukey-Kramer correction for multiple tests.


right anterior canal
gain=0.86, sacc amp=0.00°/trial
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right horizontal canal
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left horizontal canal
gain=0.29, sacc amp=4.61°/trial
right posterior canal
gain=0.41, sacc amp=3.27°/trial
left posterior canal
gain=0.41, sacc amp=1.05°/trial
head and eye velocity [°/s]
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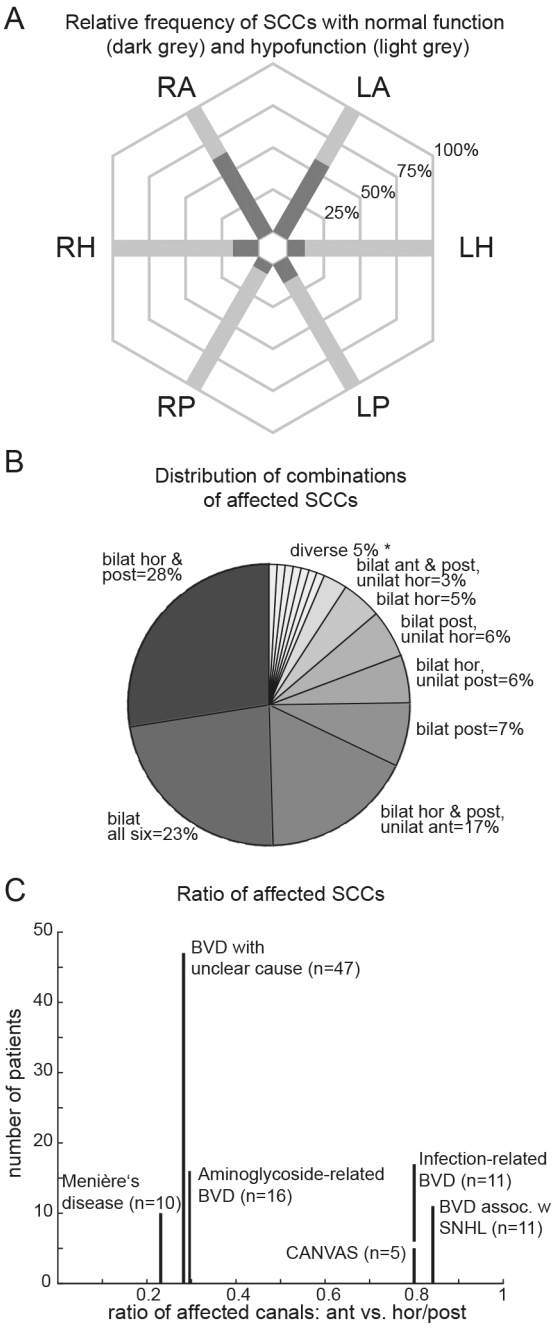
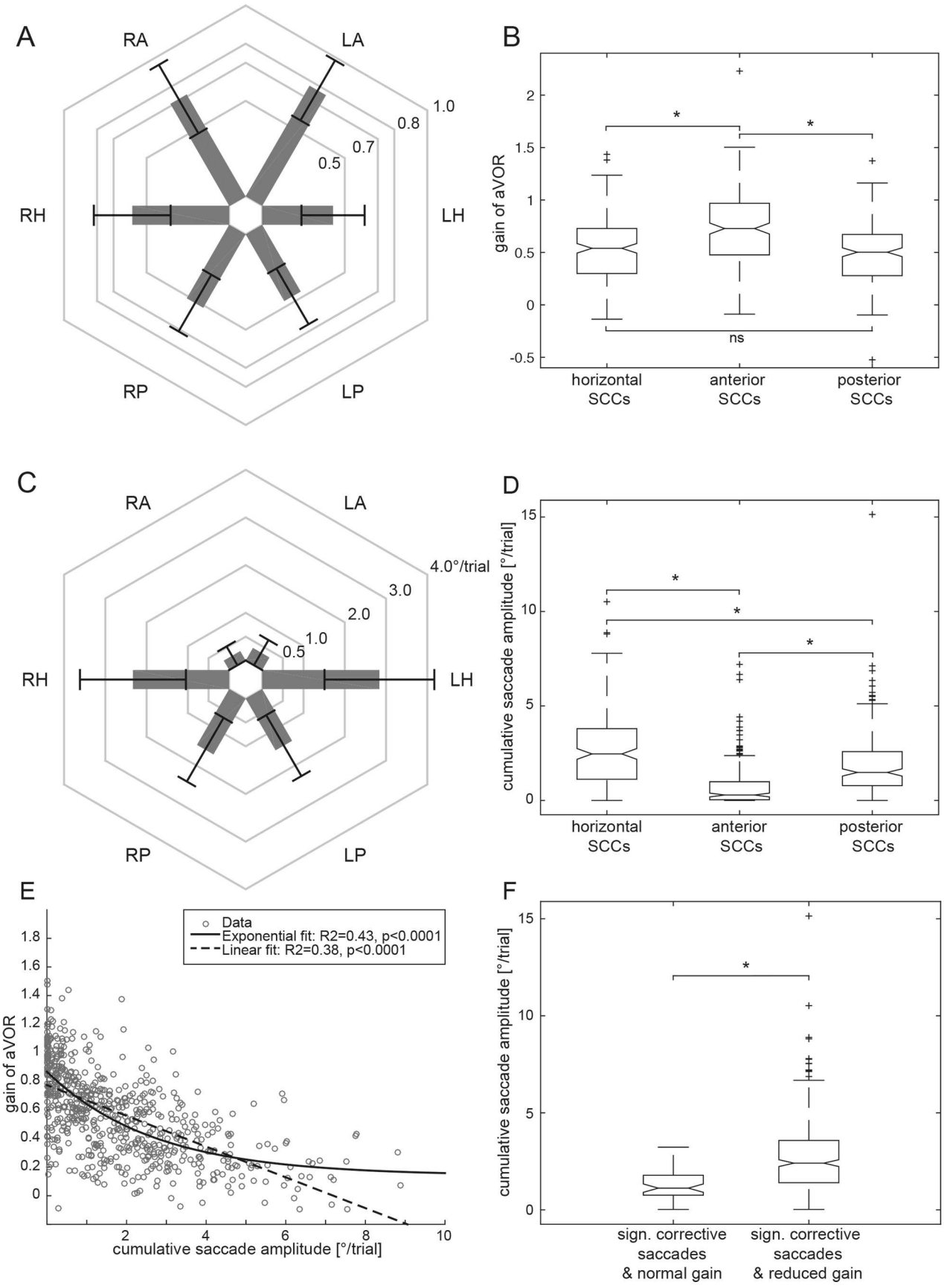
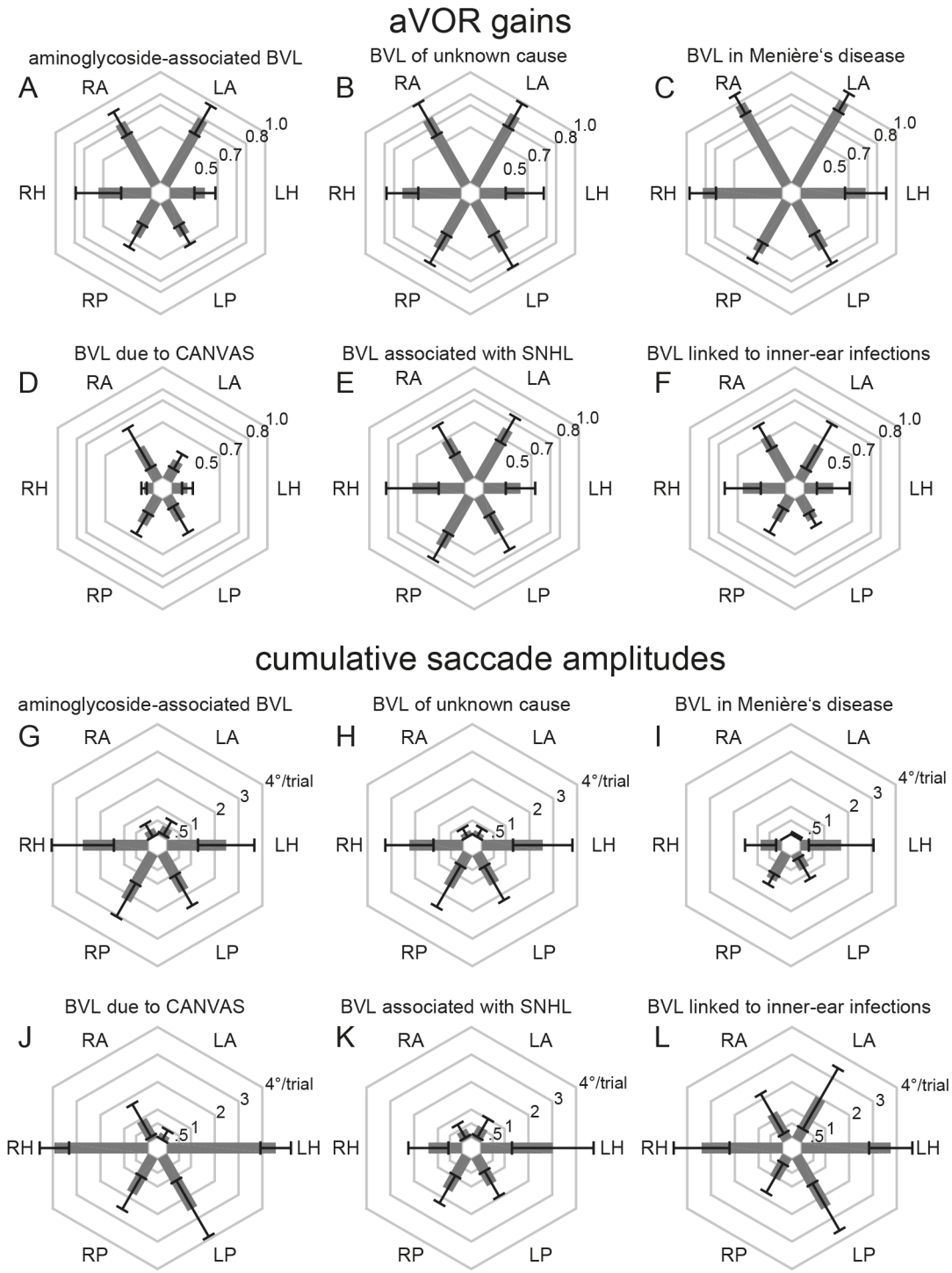
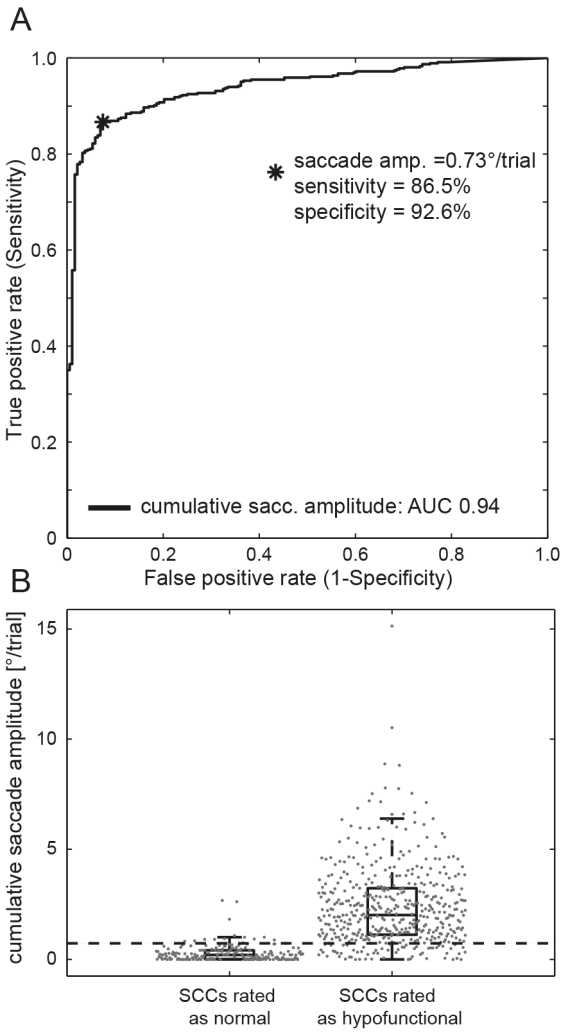

A
Relative frequency of SCCs with normal function
(dark grey) and hypofunction (light grey)
RA
LA
RH
LH
RP
LP
100%
75%
50%
25%
B
Distribution of combinations
of affected SCCs
bilat hor &
post=28%
diverse 5% *
bilat ant & post,
unilat hor=3%
bilat hor=5%
bilat post,
unilat hor=6%
bilat hor,
unilat post=6%
bilat post=7%
bilat hor & post,
unilat ant=17%
bilat
all six=23%
C
Ratio of affected SCCs
number of patients
ratio of affected canals: ant vs. hor/post
BVD with
unclear cause (n=47)
Menière's
disease (n=10)
Aminoglycoside-related
BVD (n=16)
Infection-related
BVD (n=11)
BVD assoc. w
SNHL (n=11)
CANVAS (n=5)
0
10
20
30
40
50
0.2
0.4
0.6
0.8
1


A
RA
LA
RH
LH
RP
LP
1.0
0.8
0.7
0.5
B
gain of aVOR
2
1.5
1
0.5
0
-0.5
*
*
ns
horizontal SCCs
anterior SCCs
posterior SCCs
C
RA
LA
RH
LH
RP
LP
4.0°/trial
3.0
2.0
1.0
0.5
D
cumulative saccade amplitude [°/trial]
15
10
5
0
*
*
*
horizontal SCCs
anterior SCCs
posterior SCCs
E
Data
Exponential fit: R2=0.43, p<0.0001
Linear fit: R2=0.38, p<0.0001
gain of aVOR
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
2
4
6
8
10
cumulative saccade amplitude [°/trial]
F
cumulative saccade amplitude [°/trial]
15
10
5
0
*
sign. corrective saccades & normal gain
sign. corrective saccades & reduced gain


aVOR gains
aminoglycoside-associated BVL
BVL of unknown cause
BVL in Menière's disease
BVL due to CANVAS
BVL associated with SNHL
BVL linked to inner-ear infections
A
B
C
D
E
F
RA
LA
RH
LH
RP
LP
0.5
0.7
0.8
1.0
cumulative saccade amplitudes
G
H
I
J
K
L
4°/trial
.5
1
2
3


A
1.0
0.8
0.6
0.4
0.2
0
True positive rate (Sensitivity)
saccade amp. =0.73°/trial
sensitivity = 86.5%
specificity = 92.6%
cumulative sacc. amplitude: AUC 0.94
0
0.2
0.4
0.6
0.8
1.0
False positive rate (1-Specificity)
B
cumulative saccade amplitude [°/trial]
15
10
5
0
SCCs rated as normal
SCCs rated as hypofunctional